**_N_-substituted pyrrole-based scaffolds as potential anticancer lead structures**

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## Abstract

Undoubtedly, efficient cancer treatment is a significant challenge for the scientific community the last decades. Despite tremendous progress made towards this direction, there are still efforts needed to discover new anticancer drugs. In this work, a series of *N*-substituted pyrrole-based scaffolds have been synthesized and evaluated for antiproliferative activity against a panel of cancer cell lines (L1210, CEM and HeLa). Furthermore, in order to discover new scaffolds as antivirus agents all the examined compounds were evaluated for antivirus activity against different types of DNA and RNA viruses. The key feature of the above structures is the existence of an aromatic ring with at least one hydrogen-bonding donor and acceptor group. Results have shown interesting cytostatic activity for three of the synthesized compounds (**1**, **3** and **9**). Especially, compound **1**, containing a tropolone ring, proved to be the most promising scaffold ($IC_{50}$:10-14 μM) for the development of novel potential anticancer agents. In addition, compound **1** has shown interesting antivirus activity as a scaffold against a variety of viruses.



## Keywords

*N*-substituted pyrroles, scaffolds, antiproliferative agents, antiviral activity, aromatic ring, hydrogen-bonding donor and acceptor group.


## 1. INTRODUCTION

Cancer is a leading cause of death worldwide both in developing and developed countries. It is accounted that about 7.6 million people died from cancer in 2008 and it is estimated that deaths globally are projected to continue to rise to over 13.1 million in 2030 [1]. Although there are many types of cancer, there is one defining feature in all of them - the abnormal and uncontrolled cell division. “Cancer can be caused by both external factors such as tobacco, chemicals, radiation and infectious organisms and internal factors such as inherited mutations, hormones, immune conditions and mutations that occur from metabolism”[2].

Heterocycles can serve as useful tools in medicinal chemistry to manipulate lipophilicity, polarity and hydrogen bonding capacity of molecules, which may lead to improved pharmacological, pharmacokinetic, toxicological and physicochemical properties of drug candidates and ultimately drugs [3]. Currently, a great number of heterocyclic templates are used as anticancer or antivirus agents and many efforts have been still in progress in this way. Among heterocyclic compounds, the pyrrole moiety is a prominent chemical motif, found widely in natural products, biologically important molecules, drugs and advanced materials. “This five-membered nitrogen-containing heteroaromatic ring, planar and electron-rich, is a useful recognition element in many biological contexts-forming hydrogen bonds, coordinating metals and providing stacking interactions” [4].

Thus, a series of *N*-substituted pyrrole-based scaffolds (Figure **1**) have been designed, synthesized and evaluated for their antiproliferative activity against three different cancer cell lines. Additionally, in order to obtain potent antivirus agents as scaffolds, the antiviral activity of the examined compounds were tested *in vitro*. The key structural component of the above substitutes is the existence of an aromatic ring with at least one hydrogen-bonding donor and acceptor group. “Hydrogen bonding is a directional and moderately strong intermolecular force. Compounds that present multiple hydrogen bond donor and acceptor groups have proven to be extremely important in creating new self-assembled structures” [5]. It is also known that hydrogen bonds are used to stabilize and determine the structure of macromolecules like proteins and nucleic acids, as well as they contribute to ligand recognition by biological receptors [6].

## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

Two different methodologies were followed for the synthesis of the pyrrole derivatives (Scheme **1**). The first one, employed for compounds **1-6**, utilized a modified Clauson-Kaas-type reaction for the formation of the pyrrole ring [7]. Commercially-available arylamines and 5-aminotropolone [8] were treated with 2,5-dimethoxytetrahydrofuran and 4-chloropyridinium hydrochloride as a catalyst to afford the respective compounds. Alternatively, the appropriate aryl iodides were converted to **7** and **8**, by means of a modified Ullman-type coupling reaction [9]. In this modification, 1,3-difluoro-4-iodo-2-methoxybenzene [10] and 1-(benzyloxy)-4-iodo-1H-pyrazole [11] were coupled with pyrrole, after treatment with CuI and trans-*N*,*N* -tetramethylcyclohexane-1,2-diamine as catalysts and $K_3PO_4$ as the base.

End products **9-11** were obtained after demethylation of the respective methyl ethers **5-7** with pyridinium hydrochloride [12]. In contrast, the benzyl group of compound **8** was selectively cleaved after treatment with equal amounts of Pd/C and ammonium formate at 0°C, to afford **12** [9] (Scheme **2**).

Finally, the synthesis of compounds **13** [13] and **14** [14] is described in the related articles.

### 2.2. Biological Evaluation

Compounds **1** to **4**, and **9** to **14** have been evaluated for their cytostatic activity against murine leukemia L1210, human $CD4^+$ T-lymphocyte CEM and human cervix carcinoma HeLa cells.

From the obtained results (Table **1**) it is obvious that compounds **2**, **4** and **10**-**14** presented negligible, if any antiproliferative activity ($IC_{50}$ > 100 μM), in contrast to compounds **1**, **3** and **9.** Especially, the tropolone derivative (**1**) was consistently found to be the most cytostatic in all examined cancer cell lines ($IC_{50}$: 10-14 μM) followed by **3** ($IC_{50}$: 22-33 μM) and **9** ($IC_{50}$: 32-100 μM). It was interesting to reveal the markedly lower cytostatic activity of the closely-related **4** (*versus* **3**) and **10** (*versus* **9**) derivatives. Also, **1**, **3** and **9** were not cytotoxic in normal (primary) confluent human lung fibroblast (HEL) cell cultures ($MCC_{50}$ > 100 μM). This may point to a very structure-selective interaction of the drug molecules with their cytostatic target. The cytostatic potential of **1** was only 5- to 10-fold lower than the established 6-mercaptopurine anticancer drug. It is generally accepted, that compounds with a tropolone skeleton might have a broad spectrum of biological activities such as an antimicrobial, antifungal, phytogrowth-inhibitory and cytotoxic effect on mammalian tumor cells [15]. In fact, Ononye et al, recently found that a series of tropolone derivatives was found to be potent and selective histone diacetylase inhibitors [16]. However, the molecular target of **1** is currently not known. It should also be noticed that **1** exerted a modest anti-HSV-1, -HSV-2 and –vaccinia virus activity in HEL cell cultures ($EC_{50}$: 27-40 μM) whereas none of the other compounds showed appreciable antiviral activity against a broad variety of DNA and RNA viruses (data not shown).

## 3. CONCLUSION

In summary, a series of *N*-substituted pyrrole-based scaffolds were examined for antiproliferative activity against three cancer cell lines as well as for antiviral activity. Among the studied compounds, compound **1** which has a tropolone ring, demonstrated the most potent activity against all cancer cell lines ($IC_{50}$:10-14 μM). Also, compound **3** showed pronounced cytostatic activity, while compound **9** exhibited cytostatic activity only against CEM and HeLa. Furthermore, from the examined compounds, only **1** presented to be a promising antiviral agent as scaffold. In general, the findings suggested that compounds **1**, **3** and **9** could represent interesting scaffolds for the development of novel potential anticancer agents. Further modifications of these scaffolds are currently under consideration.

## 4. EXPERIMENTAL METHODS

## 4.1. Chemistry

General: All reagents were purchased from Sigma–Aldrich Co. and used without further purification, except for the solvents used for flash chromatography and recrystallization. $^{1}$H NMR spectra were recorded on a Bruker AM 300 at 300 MHz and chemical shifts are given in d referenced to the residual solvent peak. $^{13}$C NMR spectra at 75.5 MHz on the same spectrometer, using chloroform-*d* ($CDCl_3$), dimethylsulfoxide-$d_6$ (DMSO-$d_6$). Melting points are uncorrected and were determined in open glass capillaries using a Mel-Temp II apparatus. A Shimadzu 2010-EV LC/MS was used for obtaining a LC/MS spectrum, using methanol or mixture of methanol-water (formic acid 0.1%) as solvents. All LC-MS spectras were obtained under isocratic elution in a reversed phase $C_{18}$ column. Microanalyses were performed on a Perkin-Elmer 2400-II Analyzer. Flash column chromatography was carried out on a Merck silica gel 60 plate (230–400 Mesh ASTM). Reaction progress was followed by thin-layer chromatography (Fluka Silica gel/TLC-cards). All solvents used for column chromatography and/or recrystallization were routinely distilled prior to use. Petroleum ether refers to the fraction with bp 40$^{o}$–60$^{o}$ C. The known compounds **13** and **14** were synthesized as previously described [12, 13] for the purpose of evaluation of their antiproliferative activity.

## 4.2. General Method for Synthesis of Compounds 1-6: The modified Clauson-Kaas Type Reaction

To a solution of the appropriate arylamine (29.4 mmol) in dioxane (210 ml), 2,5-dimethoxytetrahydrofuran (5.36 g, 40.6 mmol) and 4-cloropyridinium hydrochloride (6.79 g, 45.3 mmol) were added. The mixture was refluxed under a nitrogen atmosphere for 3 h. After removal of the solvent under reduced pressure, the residue was treated with 50 ml of $CH_2Cl_2$ and filtered. The filtrate was concentrated in vacuo and the residue was purified with flash column chromatography, using various solvent mixtures as eluent. In the case of **1**, after removal of the solvent under reduced pressure, the residue was treated with 50 ml of EtOAc and filtered. EtOAc was removed under reduced pressure from the filtrate and the residue was treated with 10% $NaHCO_3$ (20 ml) and extracted with EtOAc (2 x 50 ml). The aqueous phase was cooled (ice bath) and acidified with 10% HCl, and extracted with EtOAc (2 x 50 ml). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure.

### *4.2.1. 2-hydroxy-5-(1H-pyrrol-1-yl)cyclohepta-2,4,6-trienone (1)*

Yield: 63% after recrystallization by AcOEt/petroleum ether; m.p.: 184-187°C; $^{1}$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 6.20-6.29 (m, 2H), 7.04-7.09 (m, 2H), 7.22-7.31 (m, 2H), 7.43-7.56 (m, 2H), 7.99 (s, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 116.03, 124.92, 129.46, 135.31, 144.27, 175.21; LC–MS: m/z= 188 $[M+H]^+$, 186 $[M-H]^+$. Anal. Calcd. for $C_{11}H_9NO_2$ (%): C, 70,58; H, 4.85; N, 7.48. Found: C, 70.77; H, 4.48; N, 7.49.

### *4.2.2. 5-(1H-pyrrol-1-yl)-1,3,4-thiadiazole-2-thiol (2)*

Yield: 42% after flash column chromatography with petroleum ether/AcOEt mixture (10:1); m.p.: 185-187°C; $^{1}$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 6.20-6.30 (m, 2H), 6.94-7.06 (m, 2H), 7.35 (s, 1H). $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 112.85, 119.44, 153.32, 185.21; LC–MS m/z: 182 $(M-H)^+$. Analytical sample was obtained by recrystallization from $CH_2Cl_2$/petroleum ether. Anal. Calcd. for $C_6H_5N_3S_2$ (%): C, 39.32; H, 2.75; 22.93. Found: C, 39.72; H, 2.86; N, 22.81.

### *4.2.3. 5-(1H-pyrrol-1-yl)-1H-indazole (3)*

Yield: 71% after flash column chromatography with $CH_2Cl_2$; m.p.: 181-184°C; $^{1}$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 6.10-6.19 (m, 2H), 6.85-6.96 (m, 2H), 7.18-7.31 (m, 1H), 7.35-7.46 (m, 1H), 7.47-7.55 (m, 1H), 7.82-7.91 (m, 1H), 12.5 (s, br, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 109.81, 111.14, 111.77, 119.96, 121.09, 123.15, 133.74, 134.59, 138.7; LC–MS m/z= 184 $(M+H)^+$,182 $(M-H)^+$. Analytical sample was obtained by recrystallization from $CH_2Cl_2$/petroleum ether. Anal. Calcd. for $C_{11}H_9N_3$(%): C, 72.11; H, 4.95; N, 22.94. Found: C, 72.44; H, 5.13; N, 23.21.

#### *4.2.4. 6-(1H-pyrrol-1-yl)-1H-indazole (4)*

Yield: 74% after flash column chromatography with $CH_2Cl_2$; m.p.: 201-202°C; $^1$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 5.89-6.04 (m, 2H), 6.72-6.80 (m, 2H), 6.84-6.92 (m, 1H), 7.08-7.17 (m, 1H), 7.33-7.47 (m, 1H), 7.58-7.72 (m, 1H), 12.37 (s, br, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 100.87, 110.32, 114.78, 119.53, 120.66, 121.19, 132.94, 138.58, 140.23; LC–MS m/z= 184 $(M+H)^+$, 182 $(M-H)^+$. Analytical sample was obtained by recrystallization from $CH_2Cl_2$/petroleum ether. Anal. Calcd. for $C_{11}H_9N_3$ (%): C, 72.11; H, 4.95; N, 22.94. Found: C, 72.33; H, 4.89; N, 22.78.

#### *4.2.5. 6-methoxy-2-(1H-pyrrol-1-yl)benzo[d]thiazole (5)*

Yield: 70% after flash column chromatography with petroleum ether/AcOEt mixture (25:1); m.p.: 118-120°C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$= 3.89 (s, 3H), 6.40-6.45 (m, 2H), 7.03-7.12 (m, 1H), 7.24-7.32 (m, 1H), 7.40-7.46 (m, 2H), 7.79 (d, J=8.9 Hz, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$): $\delta$= 55.68, 104.73, 112.16, 114.98, 119.77, 122.47, 133.12, 145.27, 157.16; LC–MS m/z= 231 $(M+H)^+$, 254 $(M+H+Na)^+$, 229 $(M-H)^+$. Analytical sample was obtained by recrystallization from $Et_2O$/petroleum ether. Anal. Calcd. for $C_{12}H_{10}N_2OS \cdot 0.05Et_2O$(%): C, 62.62; H, 4.52; N, 11.97. Found: C, 62.89; H, 4.58; N, 12.04.

#### *4.2.6. 4-methoxy-2-(1H-pyrrol-1-yl)benzo[d]thiazole (6)*

Yield: 90% after flash column chromatography with petroleum ether/AcOEt mixture (23:1); m.p.: 88-90°C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$= 3.70 (s, 3H), 5.95-6.15 (m, 2H), 6.51-6.70 (m, 1H), 6.95-7.05 (m, 2H), 7.09-7.26 (m, 2H); $^{13}$C NMR (75.5 MHz, $CDCl_3$): $\delta$= 56.13, 107.69, 112.44, 113.45, 115.54, 120.24, 125.49, 125.88, 128.63, 133.47, 140.96, 152.71, 158.36; LC–MS m/z= 231 $(M+H)^+$, 254 $(M+H+Na)^+$, 229 $(M-H)^+$. Analytical sample was obtained by recrystallization from $Et_2O$/petroleum ether. Anal. Calcd. for $C_{12}H_{10}N_2OS \cdot 0.05Et_2O$(%): C, 62.62; H, 4.52; N, 11.97. Found: C, 62.57; H, 4.61; N, 11.59.

### 4.3. Synthesis of 1-(2,4-difluoro-3-methoxyphenyl)-1H-pyrrole (7)

To a stirred solution of 1,3-difluoro-4-iodo-2-methoxybenzene (448 mg, 1,66 mmol) in dry toluene (2 ml), pyrrole (93 mg, 1.39 mmol), trans-tetramethylcyclohexane-1,2-diamine (40 mg, 0.28 mmol) and $K_3PO_4$ (620 mg, 2.92 mmol) were added. A catalytic amount of CuI (13 mg, 0.07 mmol) was, then, suspended and the mixture was refluxed for 24 h under $N_2$ atmosphere. The reaction mixture was cooled in room temperature, diluted with 4 ml of $CH_2Cl_2$ and filtered through a plug of silica. Additional $CH_2Cl_2$ (70-90 ml) were used to wash the silica and the solvents were removed under reduced pressure. Further purification of the resulting residue was achieved by silica gel flash column chromatography, using a mixture of petroleum ether/AcOEt (50:1) as eluent and gave viscous yellowish oil. Yield: 53%; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$= 4.09 (s, 3H), 6.33-6.45 (m, 2H), 6.90-7.11 (m, 4H). $^{13}$C NMR (75.5 MHz, $CDCl_3$): $\delta$= 61.91, 109.86, 111.60, 117.83, 121.32, 126.48, 147.50, 155.34; LC–MS m/z= 210 $(M+H)^+$, 208 $(M-H)^+$.

### 4.4. General method for the synthesis of compounds 9-11: Cleavage of methyl ethers

To pyridine hydrochloride (6.94 g, 60 mmol) that had been preheated at 210°C for 10 min, the appropriate methyl ether (2.6 mmol) was added and the mixture was stirred under a nitrogen atmosphere at 210°C for 1 h. The reaction mixture was then poured into ice and the resulting solution was extracted with $Et_2O$ (3 x 50 ml). The combined organic phases were washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and removed under reduced pressure. The resulting residue was purified with flash column chromatography, using a mixture of petroleum ether/AcOEt (ratios from 12:1 to 22:1) as eluent.

#### *4.4.1. 2-(1H-pyrrol-1-yl)benzo[d]thiazol-6-ol (9)*

Compound **9** was prepared from **5**. Yield: 60%; m.p.: 217-220°C; $^1$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 6.27-6.35 (m, 2H), 6.96 (dd, J=2.4 Hz, J= 8.8 Hz, 1H), 7.17-7.24 (m, 1H), 7.29 (s, 1H), 7.32-7.40 (m, 2H), 7.64 (d, J=8.8 Hz, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 106.94, 112.13,

115.93, 119.79, 122.36, 133.06, 144.26, 154.87, 156.66; LC–MS m/z= 217 $(M+H)^+$, 215 $(M-H)^+$. Analytical sample was obtained by recrystallization from $Et_2O$/petroleum ether. Anal. Calcd. for $C_{11}H_8N_2OS$ (%): C, 61.09; H, 3.73; N, 12.95. Found: C, 61.32; H, 3.75; N, 13.05.

#### *4.4.2. 2-(1H-pyrrol-1-yl)benzo[d]thiazol-4-ol (10)*

Compound **10** was prepared from **6**. Yield: 58%; m.p.: 147-148°C; $^1$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 6.30 (s, br, 1H), 6.36-6.45 (m, 2H), 7.00 (d, J= 25.1 Hz, 1H), 7.27 (dd, J=7.9 Hz, J= 22.7 Hz, 1H), 7.40-7.48 (m, 2H); $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 111.35, 112.86, 120.04, 122.37, 125.96, 128.45, 132.21, 139.81, 149.14, 158.23; LC–MS m/z= 217 $(M+H)^+$, 240 $(M+H+Na)^+$, 215 $(M-H)^+$. Analytical sample was obtained by recrystallization from high boil petroleum ether (80-110°C). Anal. Calcd. for $C_{11}H_8N_2OS$ (%): C, 61.09; H, 3.73; N, 12.95. Found: C, 61.24; H, 3.81; N, 13.13.

#### *4.4.3. 2,6-difluoro-3-(1H-pyrrol-1-yl)phenol (11)*

Compound **11** was prepared from **7**. Yield: 62%; m.p.: 76-78°C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$= 6.33-6.38 (m, 2H), 6.85-6.91 (m, 1H), 6.93-7.00 (m, 3H), 7.26 (s, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$): $\delta$= 110.08, 114.71, 114.78, 121.40, 144.04, 145.95, 148.70, 150.64; LC–MS m/z= 194 $(M-H)^+$. Analytical sample was obtained by recrystallization from $CH_2Cl_2$/petroleum ether. Anal. Calcd. for $C_{10}H_7F_2NO$ (%): C, 61.54; H, 3.62; N, 7.18. Found: C, 61.47; H, 3.28; N, 7.13.

### 4.5. Synthesis of 4-(1H-pyrrol-1-yl)-1H-pyrazol-1-ol (12)

To a stirred solution of **8** (368 mg, 1.54 mmol) in 28 ml of dry MeOH/THF mixture (1:1), ammonium formate (542 mg, 8.6 mmol) was added and the reaction mixture was cooled to 0°C under $N_2$ atmosphere. Palladium on carbon 10% (542 mg, 5.0 mmol) was, then, suspended to the cold mixture, which was stirred further for 1h. Afterwards, the reaction mixture was diluted with 20 ml of MeOH and filtered through a Whatman filter paper, which retained the Pd/C. Additional MeOH (ca. 100 ml) was used to wash the Pd/C and the combined solvents were removed under reduced pressure. The residue was dissolved in equal parts of $H_2O$/AcOEt (200 ml), the organic layer was collected, dried over $Na_2SO_4$ and concentrated in vacuo. The resulting residue was purified by recrystallization from $CH_2Cl_2$/petroleum ether. Yield: 50%; m.p.: 162-165°C; $^1$H NMR (300 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 6.15-6.27 (m, 2H), 6.75-6.86 (m, 2H), 7.15-7.24 (m, 1H), 7.36-7.46 (m, 1H), 7.59 (s, br, 1H); $^{13}$C NMR (75.5 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$= 109.49, 114.83, 120.21, 123.43, 123.73; LC–MS m/z= 148 $(M-H)^+$. Anal. Calcd. for $C_7H_7N_3O \cdot 0.1CH_2Cl_2$ (%): C, 54.09; H, 4.60; N, 26.66. Found: C, 54.17; H, 4.61; N, 26.63.

### 4.6. Biological Methods

#### *4.6.1. Antiviral Activity Assays* **[17,18]**

"The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient ($TK^-$) HSV-1 KOS strain resistant to ACV ($ACV^r$), herpes simplex virus type 2 (HSV-2) strain G, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie virus B4, parainfluenza virus 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Reovirus-1, Sindbis virus, Punta Toro virus, human immunodeficiency virus type 1 strain $III_B$ and human immunodeficiency virus type 2 strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa) or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the $EC_{50}$ or compound concentration required to reduce virus-induced cytopathogenicity by 50%".

#### *4.6.2. Anti-HIV Activity Assays* **[17,18]**

"Inhibition of HIV-1($III_B$)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing $3 \times 10^5$ CEM cells/ml infected with 100 $CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4−5 days of incubation at 37°C in a $CO_2$-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The $EC_{50}$ (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%".

#### *4.6.3. Cytostatic Activity Assays* **[17,18]**

"All assays were performed in 96-well microtiter plates. To each well were added $(5−7.5) \times 10^4$ tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at 37°C in a humidified $CO_2$-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The $IC_{50}$ (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%".

#### *4.6.4. Cytotoxic Activity Assay* **[17,18]**

Confluent human lung fibroblast (HEL) cultures in 96-well microtiter plates were exposed to serial dilutions of the test compounds (i.e. 100, 20, 4, 0.8 µM). After 3 days of incubation at 37°C, microscopical detectable alterations of cell morphology were examined.

## 5. CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.


## 6. ACKNOWLEDGMENT

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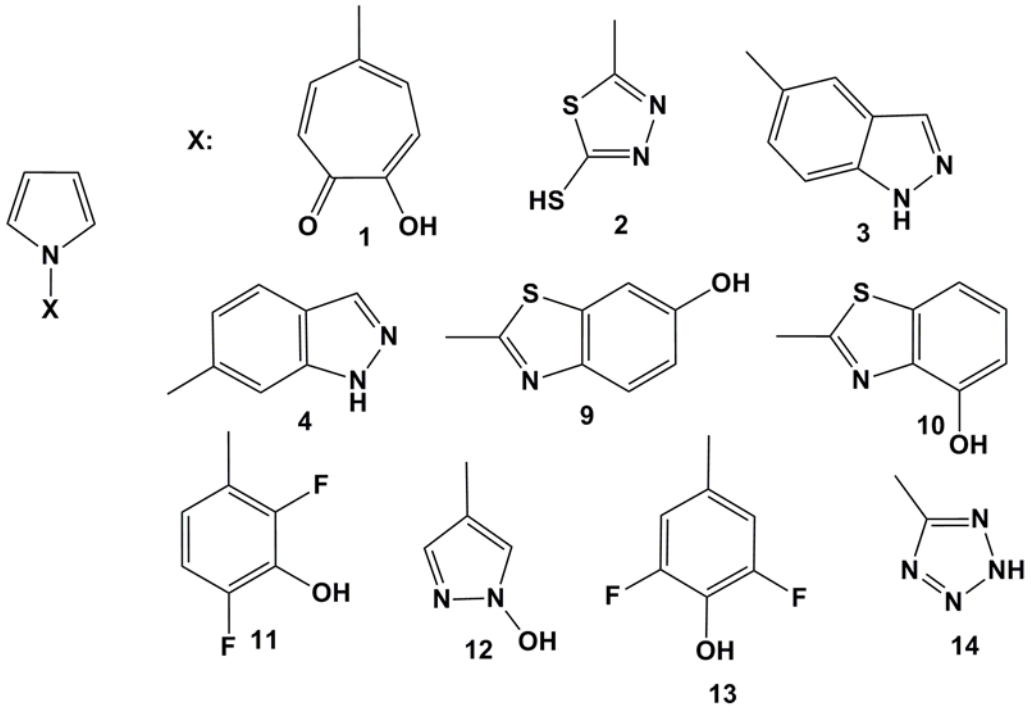
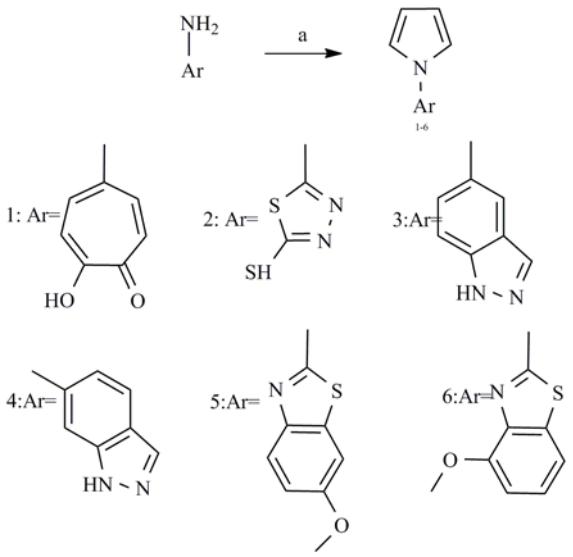
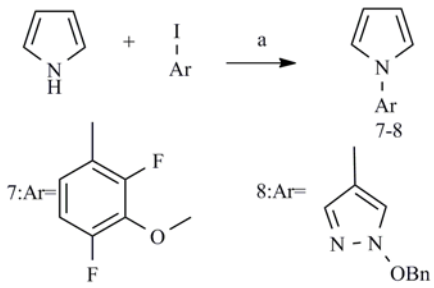


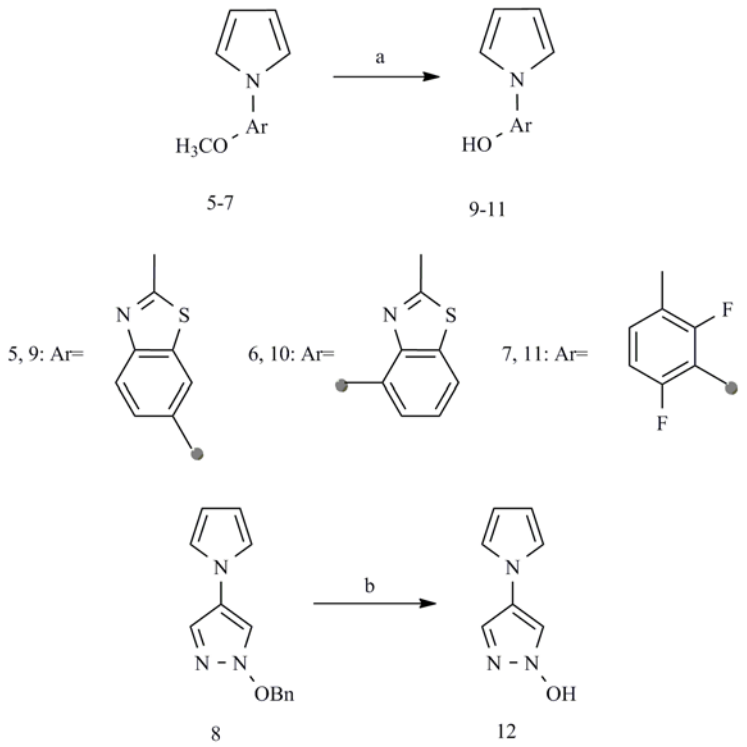

**Figure 1**: *N*-substituted pyrrole-based scaffolds




**Scheme 1**: Reagents and Conditions: (a) 2,5-dimethoxytetrahydrofuran, 4-chloropyridine hydrochloride, 1,4-dioxane, reflux, 3h.




**Scheme 2**: Reagents and Conditions: (a) $K_3PO_4$, CuI, trans-N, N'-tetramethylcyclohexane-1,2-diamine, toluene, reflux, 24h.




**Scheme 3:** Reagents and Conditions: (a) pyridine hydrochloride, 210$^{o}$C, 1h. (b) $HCOONH_4$, Pd/C, MeOH/THF, 0$^{o}$C, 1h.

**Table 1**. Antiproliferative and Cytotoxic Activity of Compounds against a Panel of Cancer Cell Lines and Primary fibroblasts

| Compound | $IC_{50}$[a] (μM) | | | MCC[b] (μM) |
|---|---|---|---|---|
| | L1210 | CEM | HeLa | HEL |
| PEG102 (**1**) | 13 ± 4 | 10 ± 1 | 14 ± 3 | >100 |
| PPN16 (**2**) | >250 | >250 | >250 | >100 |
| PPN10 (**3**) | 24 ± 3 | 33 ± 8 | 22 ± 1 | >100 |
| PPN11 (**4**) | 100 ± 8 | 107 ± 23 | 47 ± 4 | >100 |
| PPN15 (**9**) | 100 ± 2 | 49 ± 14 | 32 ± 3 | >100 |
| PPN14 (**10**) | 112 ± 1 | 104 ± 20 | 107 ± 3 | >100 |
| PPN12 (**11**) | 120 ± 2 | ≥250 | 110 ± 5 | >100 |
| PPN17 (**12**) | >250 | >250 | >250 | >100 |
| PPN13 (**13**) | 194 ± 23 | 216 ± 47 | ≥250 | >100 |
| PEG100 (**14**) | >250 | >250 | >250 | >100 |
| 5-Fluorouracil | 0.33 ± 0.17 | 18 ± 5 | 0.54 ± 0.12 | - |
| 6-Mercaptopurine | 2.8 ± 1.1 | 2.8 ± 1.3 | 1.1 ± 0.1 | - |

[a] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%. Data are the mean average of 2-3 independent experiments (± SD).

[b] Minimal cytotoxic concentration or compound concentration required to affect and alter microscopically detectable human lung fibroblast HEL cell morphology.